



SEQUENCE LISTING

<110> Bruck, Claudine

Bollen, Alex

Jacobs, Paul

Massaer, Marc

<120> Recombinant Allergen with Reduced
Enzymatic Activity

<130> B45122

<140> 09/554,860

<141> 2000-05-19

A2
<150> PCT/EP98/07521

<151> 1998-11-16

<150> GB 9724531.0

<151> 1997-11-19

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<213> Artificial Sequence

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<223> Mutant of DerP1

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gaagatgaag aagctgcccg taaaaacttt ttggaatcag taaaatatgt tcaatcaaat 180
ggagggtgcc tcaaccattt gtccgatttg tcgttggatg aattcaaaaa ccgatttttg 240
atgagtgacg aagcttttga acacctcaaa actcaattcg atttgaatgc tgaaactaac 300

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gcttgcagta tcaatggaaa tgctccagct gaaatcgatt tgcgacaaaat gcgaactgtc 360
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 actgaatcag cttatttggc ttaccgtaat caatcattgg atcttctgtga acaagaatta 480
 gtctgattgt cttcccaaca cggttgtcat ggtgatacca tccacgtgg tattgaatac 540
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 aatgtaacaa aaattcgtga agctttggct caaaccacaa gcgtatttgc cgtcattatt 720
 ggcacaaag atttagacgc attccgtcat tatgatggcc gaacaatcat tcaacgcgat 780
 aatggttacc aacaaaacta tgctgctgtc aacattgttg gttacagtaa cgcacaaggt 840
 gtcgattatt ggatcgtacg aaacagttgg gataccaatt ggggtgataa tggttacggg 900
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 20 25 30
 Phe Asn Lys Ser Tyr Ala Thr Phe Glu Asp Glu Glu Ala Ala Arg Lys
 35 40 45
 Asn Phe Leu Glu Ser Val Lys Tyr Val Gln Ser Asn Gly Gly Ala Ile
 50 55 60
 Asn His Leu Ser Asp Leu Ser Leu Asp Glu Phe Lys Asn Arg Phe Leu
 65 70 75 80
 Met Ser Ala Glu Ala Phe Glu His Leu Lys Thr Gln Phe Asp Leu Asn
 85 90 95
 Ala Glu Thr Asn Ala Cys Ser Ile Asn Gly Asn Ala Pro Ala Glu Ile
 100 105 110
 Asp Leu Arg Gln Met Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly
 115 120 125
 Cys Gly Ser Ala Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala
 130 135 140

Tyr Leu Ala Tyr Arg Asn Gln Ser Leu Asp Leu Ala Glu Gln Glu Leu
 145 150 155 160
 Val Asp Cys Ala Ser Gln His Gly Cys His Gly Asp Thr Ile Pro Arg
 165 170 175
 Gly Ile Glu Tyr Ile Gln His Asn Gly Val Val Gln Glu Ser Tyr Tyr
 180 185 190
 Arg Tyr Val Ala Arg Glu Gln Ser Cys Arg Arg Pro Asn Ala Gln Arg
 195 200 205
 Phe Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asn Val Asn Lys
 210 215 220
 Ile Arg Glu Ala Leu Ala Gln Thr His Ser Ala Ile Ala Val Ile Ile
 225 230 235 240
 Gly Ile Lys Asp Leu Asp Ala Phe Arg His Tyr Asp Gly Arg Thr Ile
 245 250 255
 Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr Ala Ala Val Asn Ile
 260 265 270
 Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile Val Arg Asn
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tatttggtt accgtaatca atcattggat ctgtctgaac aagaattagt cgattgtgct 480
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 ggtgtcgtcc aagaaagcta ctatcgatac gttgcacgag aacaatcatg ccgacgacca 600
 aatgcacaac gtttcgggat ctcaaactat tgccaaattt acccaccaaa tgtaaacaaa 660
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 Tyr Ala Arg Pro Ser Ser Ile Lys Thr Phe Glu Glu Tyr Lys Lys Ala
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 Phe Asn Lys Ser Tyr Ala Thr Phe Glu Asp Glu Glu Ala Ala Arg Lys
 35 40 45
 Asn Phe Leu Glu Ser Val Lys Tyr Val Gln Ser Asn Gly Gly Ala Ile
 50 55 60
 Asn His Leu Ser Asp Leu Ser Leu Asp Glu Phe Lys Asn Arg Phe Leu
 65 70 75 80
 Met Ser Ala Glu Ala Phe Glu His Leu Lys Thr Gln Phe Asp Leu Asn
 85 90 95
 Ala Cys Ser Ile Asn Gly Asn Ala Pro Ala Glu Ile Asp Leu Arg Gln
 100 105 110
 Met Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly Cys Gly Ser Cys
 115 120 125
 Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala Tyr Leu Ala Tyr
 130 135 140
 Arg Asn Gln Ser Leu Asp Leu Ala Glu Gln Glu Leu Val Asp Cys Ala
 145 150 155 160
 Ser Gln His Gly Cys His Gly Asp Thr Ile Pro Arg Gly Ile Glu Tyr

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 165 | | 170 | | 175 | | | | | | | | | | |
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| | 180 | | | | | | 185 | | | | | | 190 | | |
| Arg | Glu | Gln | Ser | Cys | Arg | Arg | Pro | Asn | Ala | Gln | Arg | Phe | Gly | Ile | Ser |
| | 195 | | | | | | 200 | | | | | | 205 | | |
| Asn | Tyr | Cys | Gln | Ile | Tyr | Pro | Pro | Asn | Val | Asn | Lys | Ile | Arg | Glu | Ala |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Leu | Ala | Gln | Thr | His | Ser | Ala | Ile | Ala | Val | Ile | Ile | Gly | Ile | Lys | Asp |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Leu | Asp | Ala | Phe | Arg | His | Tyr | Asp | Gly | Arg | Thr | Ile | Ile | Gln | Arg | Asp |
| | | | 245 | | | | | | 250 | | | | | 255 | |
| Asn | Gly | Tyr | Gln | Pro | Asn | Tyr | His | Ala | Val | Asn | Ile | Val | Gly | Tyr | Ser |
| | 260 | | | | | | 265 | | | | | | 270 | | |
| Asn | Ala | Gln | Gly | Val | Asp | Tyr | Trp | Ile | Val | Arg | Asn | Ser | Trp | Asp | Thr |
| | 275 | | | | | | 280 | | | | | 285 | | | |
| Asn | Trp | Gly | Asp | Asn | Gly | Tyr | Gly | Tyr | Phe | Ala | Ala | Asn | Ile | Asp | Leu |
| | 290 | | | | 295 | | | | | | | 300 | | | |
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gaagatgaag aagctgcccg taaaaacttt ttggaatcag taaaatatgt tcaatcaaat 180
ggagggtgcc tcaaccattt gtccgatttg tcgttggtg aattcaaaaa ccgatttttg 240
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gcctgcagta tcaatggaaa tgctccagct gaaatcgatt tgcgacaaat gcgaactgtc 360
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actgaatcag cttatttggc ttaccgtaat caatcattgg atcttgctga acaagaatta 480
gtcggatttg cttccaaca cggttgtcat ggtgatacca ttccactgtg tattgaatac 540
atccaacata atgggtgtcgt ccaagaaagc tactatcgat acgttgcacg agaacaatca 600

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 aatggttacc aaccaaacta tgctgctgc aacattgttg gttacagtaa cgcacaaggt 840
 gtcgattatt ggatcgtacg aaacagttgg gataccaatt ggggtgataa tggttacggt 900
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<211> 320

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 Phe Asn Lys Ser Tyr Ala Thr Phe Glu Asp Glu Glu Ala Ala Arg Lys
 35 40 45
 Asn Phe Leu Glu Ser Val Lys Tyr Val Gln Ser Asn Gly Gly Ala Ile
 50 55 60
 Asn His Leu Ser Asp Leu Ser Leu Asp Glu Phe Lys Asn Arg Phe Leu
 65 70 75 80
 Met Ser Ala Glu Ala Phe Glu His Leu Lys Thr Gln Phe Asp Leu Asn
 85 90 95
 Ala Glu Thr Asn Ala Cys Ser Ile Asn Gly Asn Ala Pro Ala Glu Ile
 100 105 110
 Asp Leu Arg Gln Met Arg Thr Val Thr Pro Ile Arg Met Gln Gly Gly
 115 120 125
 Cys Gly Ser Ala Trp Ala Phe Ser Gly Val Ala Ala Thr Glu Ser Ala
 130 135 140
 Tyr Leu Ala Tyr Arg Asn Gln Ser Leu Asp Leu Ala Glu Gln Glu Leu
 145 150 155 160
 Val Asp Cys Ala Ser Gln His Gly Cys His Gly Asp Thr Ile Pro Arg
 165 170 175
 Gly Ile Glu Tyr Ile Gln His Asn Gly Val Val Gln Glu Ser Tyr Tyr

| | | |
|---|-----|-----|
| 180 | 185 | 190 |
| Arg Tyr Val Ala Arg Glu Gln Ser Cys Arg Arg Pro Asn Ala Gln Arg | | |
| 195 | 200 | 205 |
| Phe Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asn Val Asn Lys | | |
| 210 | 215 | 220 |
| Ile Arg Glu Ala Leu Ala Gln Thr His Ser Ala Ile Ala Val Ile Ile | | |
| 225 | 230 | 235 |
| Gly Ile Lys Asp Leu Asp Ala Phe Arg His Tyr Asp Gly Arg Thr Ile | | |
| | 245 | 250 |
| Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr Ala Ala Val Asn Ile | | |
| 260 | 265 | 270 |
| Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile Val Arg Asn | | |
| 275 | 280 | 285 |
| Ser Trp Asp Thr Asn Trp Gly Asp Asn Gly Tyr Gly Tyr Phe Ala Ala | | |
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| Asn Ile Asp Leu Met Met Ile Glu Glu Tyr Pro Tyr Val Val Ile Leu | | |
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<400> 7

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| 20 | 25 | 30 |
| Ala His Ser Ala Phe Ala Ala Asp Pro Arg Pro Ser Ser Ile Lys Thr | | |
| 35 | 40 | 45 |
| Phe Glu Glu Tyr Lys Lys Ala Phe Asn Lys Ser Tyr Ala Thr Phe Glu | | |
| 50 | 55 | 60 |
| Asp Glu Glu Ala Ala Arg Lys Asn Phe Leu Glu Ser Val Lys Tyr Val | | |
| 65 | 70 | 75 |
| Gln Ser Asn Gly Gly Ala Ile Asn His Leu Ser Asp Leu Ser Leu Asp | | |
| 85 | 90 | 95 |

Glu Phe Lys Asn Arg Phe Leu Met Ser Ala Glu Ala Phe Glu His Leu
 100 105 110
 Lys Thr Gln Phe Asp Leu Asn Ala Cys Ser Ile Asn Gly Asn Ala Pro
 115 120 125
 Ala Glu Ile Asp Leu Arg Gln Met Arg Thr Val Thr Pro Ile Arg Met
 130 135 140
 Gln Gly Gly Cys Gly Ser Cys Trp Ala Phe Ser Gly Val Ala Ala Thr
 145 150 155 160
 Glu Ser Ala Tyr Leu Ala Tyr Arg Asn Gln Ser Leu Asp Leu Ala Glu
 165 170 175
 Gln Glu Leu Val Asp Cys Ala Ser Gln His Gly Cys His Gly Asp Thr
 180 185 190
 Ile Pro Arg Gly Ile Glu Tyr Ile Gln His Asn Gly Val Val Gln Glu
 195 200 205
 Ser Tyr Tyr Arg Tyr Val Ala Arg Glu Gln Ser Cys Arg Arg Pro Asn
 210 215 220
 Ala Gln Arg Phe Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asn
 225 230 235 240
 Ala Asn Lys Ile Arg Glu Ala Leu Ala Gln Thr His Ser Ala Ile Ala
 245 250 255
 Val Ile Ile Gly Ile Lys Asp Leu Asp Ala Phe Arg His Tyr Asp Gly
 260 265 270
 Arg Thr Ile Ile Gln Arg Asp Asn Gly Tyr Gln Pro Asn Tyr His Ala
 275 280 285
 Val Asn Ile Val Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile
 290 295 300
 Val Arg Asn Ser Trp Asp Thr Asn Trp Gly Asp Asn Gly Tyr Gly Tyr
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 Phe Ala Ala Asn Ile Asp Leu Met Met Ile Glu Glu Tyr Pro Tyr Val
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 Val Ile Leu

<210> 8

<211> 343

<212> PRT

<213> Artificial Sequence

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35 40 45
Phe Glu Glu Tyr Lys Lys Ala Phe Asn Lys Ser Tyr Ala Thr Phe Glu
50 55 60
Asp Glu Glu Ala Ala Arg Lys Asn Phe Leu Glu Ser Val Lys Tyr Val
65 70 75 80
Gln Ser Asn Gly Gly Ala Ile Asn His Leu Ser Asp Leu Ser Leu Asp
85 90 95
Glu Phe Lys Asn Arg Phe Leu Met Ser Ala Glu Ala Phe Glu His Leu
100 105 110
Lys Thr Gln Phe Asp Leu Asn Ala Glu Thr Asn Ala Cys Ser Ile Asn
115 120 125
Gly Asn Ala Pro Ala Glu Ile Asp Leu Arg Gln Met Arg Thr Val Thr
130 135 140
Pro Ile Arg Met Gln Gly Gly Cys Gly Ser Ala Trp Ala Phe Ser Gly
145 150 155 160
Val Ala Ala Thr Glu Ser Ala Tyr Leu Ala Tyr Arg Asn Gln Ser Leu
165 170 175
Asp Leu Ala Glu Gln Glu Leu Val Asp Cys Ala Ser Gln His Gly Cys
180 185 190
His Gly Asp Thr Ile Pro Arg Gly Ile Glu Tyr Ile Gln His Asn Gly
195 200 205
Val Val Gln Glu Ser Tyr Tyr Arg Tyr Val Ala Arg Glu Gln Ser Cys
210 215 220
Arg Arg Pro Asn Ala Gln Arg Phe Gly Ile Ser Asn Tyr Cys Gln Ile
225 230 235 240
Tyr Pro Pro Asn Ala Asn Lys Ile Arg Glu Ala Leu Ala Gln Thr His
245 250 255
Ser Ala Ile Ala Val Ile Ile Gly Ile Lys Asp Leu Asp Ala Phe Arg
260 265 270
His Tyr Asp Gly Arg Thr Ile Ile Gln Arg Asp Asn Gly Tyr Gln Pro
275 280 285
Asn Tyr His Ala Val Asn Ile Val Gly Tyr Ser Asn Ala Gln Gly Val

| | | |
|---|-----|---------|
| 290 | 295 | 300 |
| Asp Tyr Trp Ile Val Arg Asn Ser Trp Asp Thr Asn Trp Gly Asp Asn | | |
| 305 | 310 | 315 320 |
| Gly Tyr Gly Tyr Phe Ala Ala Asn Ile Asp Leu Met Met Ile Glu Glu | | |
| 325 | 330 | 335 |
| Tyr Pro Tyr Val Val Ile Leu | | |
| 340 | | |

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<211> 78

<212> DNA

<213> Artificial Sequence

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<223> Primer

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<211> 74

<212> DNA

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<223> Primer

<400> 13

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<212> DNA

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<210> 15

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<210> 17
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<210> 18
<211> 114
<212> DNA
<213> Artificial Sequence

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<223> Primer

<400> 18

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tgggagtgac agttcgcat tgtcgcaaat cgatttcagc tggagcattt ccat 114

<210> 19

<211> 75

<212> DNA

<213> Artificial Sequence

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<400> 19

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atttgaacgc ctgca 75

<210> 20

<211> 67

<212> DNA

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<210> 21

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 21

catgaaaatt gttttggcca tcgcc 25

<210> 22
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 22
cgggtttttga attcatccaa cgac

24

<210> 23
<211> 78
<212> DNA
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<400> 23
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<220>
<223> Primer

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tttcatggta 70

<210> 25
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<223> Primer

<400> 25

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<210> 26

<211> 172

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 26

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<212> DNA

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<220>

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<210> 31

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Asn Ala Glu Thr

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